CREATINE KINASE (MB)
(KINETIC)
4 + 1

INTENDED USE

Vitro CPK (MB) reagent is an immunoinhibition assay intended for the in vitro quantitative determination of Creatine Kinase (CK-MB) in human serum and plasma.

METHOD

Kinetic UV method according to IFCC specifications.

Liquid stable reagent.

BACKGROUND

Creatine kinase (ATP: Creatine N-phosphotransferase, EC2.7.3.2) is a dimeric enzymecomposed of two types of monomer subunits, M (Muscular) and B (Brain). The subunits combine to form three distinct CK isoenzymes, CK-BB (CK-1), CK-MB (CK-2) and CK-MM (CK-3). CK-MM is the predominant form of CK in skeletal muscle. CK-BB is found in brainand smooth muscle. CK-MB is found in a high concentration in the myocardium (between 14 and 42%) and to a lesser extent skeletal muscle. In the absence of disease, most CKactivity in serum is due to the CK-MM isoform. Damage to the myocardium, as will occur in acute myocardial infarction (AMI), will result in increased circulating levels of the CK-MB isoform. Typically CK-MB levels become elevated 4 to 6 hours after the onset of chest pain, peak between 12 to 24 hours and return to a baseline within 48 hours. Determination of CK-MB usually on admission and at 6 hours, 12 hours, and 24 hours later, is recommended when AMI is suspected. Myocardial damage is very likely when the total CK activity is above 190 U/l, the CK-MB activity is above 24 U/l (+37°C) and the CK-MB activity fraction exceeds 6% of the total.

ASSAY PRINCIPLE

Creatine Kinase is a dimer. Its monomeric subunits are designated M (muscle) and B (brain, nerve cells). The subunits combine to form three isoenzymes namely CK-BB, CK-MB and CK-MM. The reagent contains a monoclonal antibody mix to the CK-M monomer and so completely inhibits the activity of CK-MM and one half the activity of CK-MB. The activity of the non inhibited B monomer subunit of CK-MB is measured which represents half the activity of CK-MB. The method assumes that the activity of CK-BB isoenzyme in serum is essentially zero.In this method serum is added to a modified CK-NAC reagent which contains the anti M antibody.

1. CK-MM + Antibody ————> Inhibited CK-MM
   CK-MB + Antibody ————> 50% Inhibited CK-MB
2. Inactivated CK-B ————> NAC ————> Activated CK-B

The activity of the CK-B is determined using the following series of reactions:-

Creatine phosphate + ADP $\xrightleftharpoons{\text{CK (AMP, NAC)}}$ **Creatine + ATP**

ATP + Glucose $\xrightleftharpoons{\text{HK}}$ **Glucose-6-phosphate + ATP**

G6P+NADPH+H_2O $\xrightleftharpoons{\text{G6PD}}$ **Gluconate-6P + NADPH**

The rate of reduction of the coenzyme NADP is proportional to the CPK activity in the specimen. It is determined by measuring the increase in absorbance at 334 / 340 / 365 nm correspondingly.

EXPECTED VALUES

Normal < 24 U/L

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes, the ALT results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

REAGENTS

R_1	Imidazol buffer pH 6.7	100 mmol/l
	Glucose	20 mmol/l
	NAC	20 mmol/l
	Magnesium acetate	10 mmol/l
	NADP	2.5 mmol/l
	Hexokinase	4 KU/l
	EDTA	2 mmol/l
R_2	Creatine phosphate	30 mmol/l
	ADP	2 mmol/l
	Diadenosine-5'pentaphosphate	10 µmol/l
	G6PDH	1.5 KU/l
	CK-M inhibiting polyclonal antibodies. Inhibiting Capacity	2000 U/L

- **Reagent Preparation & Stability**

All reagents are ready for use and stable up to the expiry date given on label when stored at 2–8°C.

Mix 4 ml of R_1 + 1 ml of R_2

Mix well, do not shake. the working solution is stable for:

24 days at 20–25 °C.
2 weeks at 22 – 8 °C

SPECIMEN

Serum is the only acceptable material.

EDTA or heparinized plasma. Avoid hemolysis can produce erroneous results.

Prior to the CK-MB assay, the total CK activity should be determined by the CK NAc method. The antibody is capable of inhibiting up to 2000 U/I CK-M subunit (37°C). Accordingly, CK-MM activities up to1000 U/I (37°C) are completely inhibited. Therefore, samples with total CK activities above 1000 U/I (37°C) require dilution because complete inhibition is no longer assured.

- **Specimen Preparation & Stability**

Separate serum from clot/cells immediately. CPK is stable for 8 days at 2 – 8°C or one month if stored at -20°C

PROCEDURE

- **Manual Procedure**

Wavelength 340 nm
Cuvette 1 cm light path
Temperature 25, 30 or 37 °C
Zero adjustment against air

Pipette into test tube or cuvette	
	Test
Working solution	1 ml
Serum	40 µl

Mix, and incubate for 5 minutes. Read read intial absorbance after 5 minutes the absorbance A1. Incubate again for 5 minutes and read the absorbance A2.

- **Automated Procedure**

User defined parameters for different auto analyzers are available upon request.

CALCULATION

ΔA = A2 – A1 (for 5 minutes)
CK-MB (U/l) = ΔA X Factor

Factor for 340 nm: 1651

Factor for 334 nm: 1683

Factor for 365 nm: 2972

$$\text{Factor} = \frac{TV \times 1000}{{}^{*}\Sigma \times SV \times LP} \times 2$$

Where:

TV	Total reaction volume in ml
SV	Sample volume in ml
$^{*}\Sigma$	millimolar absorptivity of NADH
LP	Cuvette pathlength in cm
1000	Conversion of U/ml to U/l
2	Multiplication of the CK-MB value by 2 gives an estimation of the CK-MB activity as only half the activity is measured.

millimolar absorptivity of NADH

at 334 nm= 6.18,

at 340 nm= 6.22, and

at 365 nm= 6.40

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- **Unit conversion**

U/l x 0.01667 x 10^{-3} = µkat/l

QUALITY CONTROL

It is recommended that controls (normal and abnormal) be included in:

- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commercially available control material with established CPK values may be routinely used for quality control.

Failure to obtain the proper range of values in the assay of control material may indicate:

- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

The following corrective actions are recommended in such situations:

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Vitro Technical Services.

INTERFERING SUBSTANCES

- **Anticoagulants:**
  Fluoride and citrate inhibit the enzyme activity. The only accepted anticoagulants are heparin and EDTA.
- **Bilirubin:**
  No interference from free bilirubin up to a level of 15 mg/dl, and from conjugated bilirubin up to level of 6.8 mg/dl.
- **Drugs:**
  Young[7] in 1990 has published a comprehensive list of drugs and substances which may interfere with this assay.
- **Haemolysis:**
  Erythrocyte contamination may elevates results, since CPK activities in erythrocytes are three to five times higher than those in normal sera.
- **Lipemia:**
  Lipemic specimens may cause high absorbance flagging. Choose diluted sample treatment for automatic rerun.

WARNING & PRECAUTION

- Vitro CPK reagent is for in vitro diagnostic use only. Normal precautions exercised in handling laboratory reagents should be followed.
- Warm up working solution to the corresponding temperature before use.
- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- Valid results depend on an accurately calibrated instrument, timing, and temperature control.

PERFORMANCE CHARACTERISTICS

Imprecision

Reproducibility was determined using in an internal protocol. The following results were obtained.

	Within Run		Between Day	
Control	Level I	Level II	Level I	Level II
Number of samples	40	40	40	40
Mean (U/l)	37	155	37	155
SD (U/l)	1.4	2.5	1.4	3.2
CV (%)	4.8	1.8	3.4	2.2

Method Comparison

Comparison studies were carried out using another similar commercially available CK-MB reagent as a reference. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained:

Number of sample pairs 45
Range of sample results 5 - 226 U/l
Mean of reference method results 44 U/l
Mean of CK-MB results 45 U/l
Slope 0.997
Intercept 1.9 U/l
Correlation coefficient 0.9995

Sensitivity

The sensitivity is defined as the lower detection limit represents the lowest measurable CPK activity that can be distinguished from zero.

When run as recommended the sensitivity of this assay is 4 U/l.

Linearity

When run as recommended, the assay is linear up to 1000 U/l.

If result exceeds 1000 U/l, specimen should be diluted 1+5 with 0.9% NaCl solution and reassayed. Multiply the result by 6.

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SYMBOL DECLARATION

	Manufacturer
	Consult instructions for use
LOT	Batch code (Lot #)
REF	Catalog number
	Temperature limitation
IVD	In vitro diagnostic medical device
	Use by
	Caution. Consult instructions
	Keep away from light

ORDERING INFORMATION

REF	SIZE
1141	5 X 10 ml
1142	10 X 10 ml
1143	4 X 20 ml
1144	3 X 50 ml
1145	10 X 20 ml

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